Purification and initial studies on vitellin/vitellogenin, an allergen in German cockroach

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Abstract

Rationale: German cockroach (*Blattella germanica)*) is a source of important urban indoor aeroallergens, associated with allergic rhinitis and asthma. Vitellogenin, a large abundant protein in *B. germanica* egg cases (oothecae), was purified and assessed for its allergenicity.
Methods: To identify vitellogenin, extracts made from whole body acetone-defatted German cockroach (GCr) were purified by ammonium sulfate precipitation and size exclusion chromatography, and extracts from cockroach egg cases were purified by size exclusion chromatography alone. SDS-PAGE (one- and two-dimensional) and liquid chromatography high-resolution mass spectrometry (LC-HRMS) analyses were performed to determine the presence of vitellogenin. IgE-ELISA and immunoblots, using sera from individuals with confirmed allergy to German cockroach, were used to assess allergenicity.
Results: Vitellogenin was successfully identified in German cockroach whole body samples by LC-HRMS and was reactive to cockroach-allergic patient sera in ELISA and immunoblot experiments. Fragments of vitellogenin were seen in many of the bands and spots present on the SDS-PAGE gels, in addition to the 100 kDa band where one fragment had been previously identified. We partially purified vitellogenin from both whole bodies and egg cases. About one-third of patient sera had IgE binding to vitellogenin from egg case samples.
Conclusion: Vitellogenin is an abundant German cockroach protein that is a candidate allergen. Further work is needed to determine the percentage of IgE that specifically binds vitellogenin in cockroach-allergic patients, and the importance of vitellogenin in the pathobiology of German cockroach allergy.

Introduction

German cockroach (GCr; *Blattella germanica*) is an important source of indoor allergens associated with allergic rhinitis and asthma. GCr allergen extracts are not standardized, and have not been characterized for allergen protein content. In our initial proteomic screen of GCr allergen extracts and source materials, we identify vitellin/vitellogenin as a candidate allergen. We confirm these findings using sera from GCr-allergic individuals, and purify GCr vitellin from GCr egg cases, as well as from conventional defatted GCr source materials.

Materials and Methods

Proteins extracted from egg cases were separated using Polyacrylamide P-60 size exclusion beads. Seroprevalence was completed by IgE-ELISA, where absorbance values were log-transformed and values greater than 95% confidence interval (CI) upper limit (UL) were considered positive. Proteins extracted from defatted GCr powder (Greer) were fractionated by ammonium sulfate precipitation, and the 60-70% saturation fraction was further separated by Sephacryl S-200 size exclusion beads and anion exchange chromatography DEAE column. SDS-PAGE (one- and two-dimensional), liquid chromatography high-resolution mass spectrometry (LC-HRMS), and immunoblots were also used for analysis. GCr-allergic sera were obtained either from PlasmaLab International or from the Inner City Asthma Consortium.[1]

Results and Discussion

German cockroach commercial extract protein profile

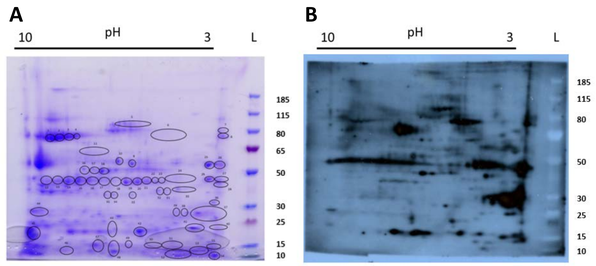

Figure 1. Coomassie-stained 2D gel electrophoresis of commercial GCr extract (Allermed) proteins and immunoblot using pooled sera from cockroach-allergic patients. The proteins were separated by 2-DE and in (A) stained with Coomassie and immunoreactive spots identified and labeled, and (B) transferred to a PVDF membrane then exposed to pooled sera from 10 cockroach allergic individuals*.
*Sera were purchased from PlasmaLab International

Vitellin purification from GCr egg cases

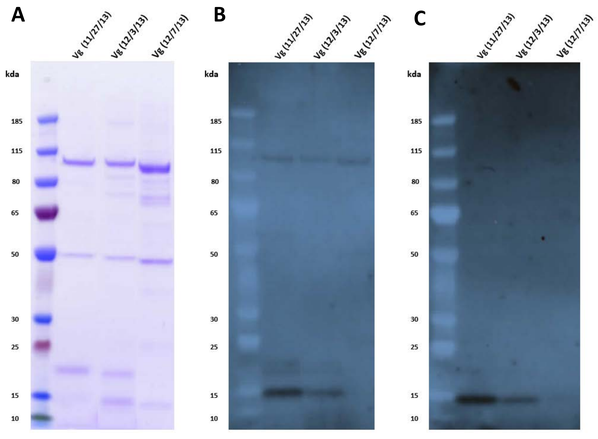

Figure 2. Coomassie-stained SDS-PAGE of purified GCr egg cases proteins and immunoblot using pooled sera from cockroach-allergic patients. The proteins were separated by SDS-PAGE and (A) stained with Coomassie, transferred to a PVDF membrane and exposed to pooled sera from (B) 10 cockroach-allergic individuals* and (C) 5 nonallergic individuals*.
*Sera were purchased from PlasmaLab International

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Seroprevalence tests on vitellin

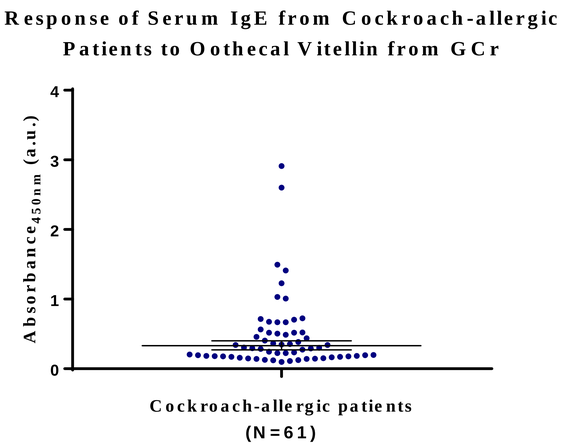

Figure 3. Seroprevalence determination of vitellin isolated from GCr egg cases, by IgE-ELISA against 61 cockroach-allergic patients[1]. 22/61 showed reactivity to vitellin. 95% confidence interval was calculated based on log-transformed values. Absorbance values greater than the UL (top line) were considered positive.

Vitellin/vitellogenin purification from defatted GCr powder: Size Exclusion Chromatography

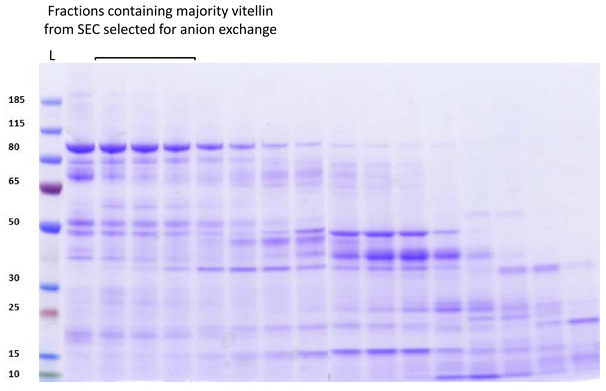

Figure 4. Coomassie-stained SDS-PAGE of proteins from defatted GCr powder after ammonium sulfate precipitation and SEC with Sephacryl S-200.

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Vitellin/vitellogenin purification from defatted GCr powder: Ion Exchange Chromatography

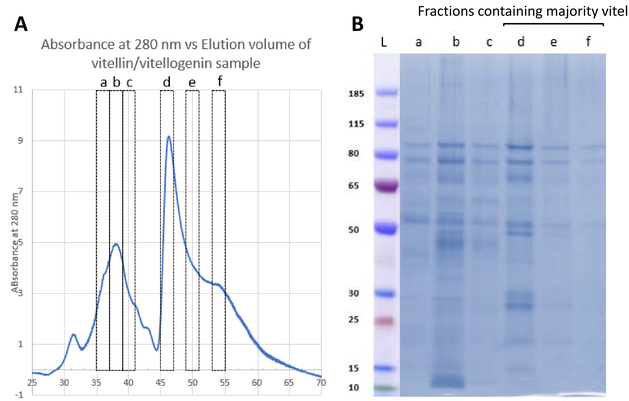

Figure 5. Separation of proteins from defatted GCr powder after ammonium sulfate precipitation, SEC, and then run over an ion exchange chromatography MacroPrep DEAE column and in (A) the chromatogram from ion exchange and (B) Silver-stained SDS-PAGE of proteins from selected fractions on chromatogram.

Conclusion

- Our analysis of commercial GCr extracts shows a large contribution of vitellin/vitellogenin to the 2D gel electrophoresis/immunoblot profiles (Figure 1).
- We performed purifications of vitellin using (a) size exclusion chromatography of GCr egg cases (Figure 2) and (b) vitellin/vitellogenin using ammonium sulfate precipitation followed by size exclusion chromatography (Figure 4) and then ion exchange chromatography of defatted GCr powder (Figure 5).
- Our analysis showed that 22 of 61 cockroach-allergic patients had IgE that recognized vitellin isolated from egg cases (Figure 3).
- Our purification from defatted GCr powder suggests a multi-step approach using ammonium sulfate precipitation followed by SEC and IEX.
- We used mass spectrometry to evaluate sample complexity and confirm vitellin/vitellogenin at various steps in the purification process.
- **Future studies:**
- Enhanced purification of vitellin and vitellogenin to greater than 95% from defatted GCr powder.
- Use pure vitellin to demonstrate IgE reactivity with inhibition experiments.

References

1.Slater JE, James R *et al*. Biological potency of German cockroach allergen extracts determined in an inner city population. J Allergy Clin Immunol 2007; 137:1033–1039